REMARKS/ARGUMENTS

Claims 1-3, 10, and 12-19 remain pending in this application, and are rejected. Claims 4-9, 11, and 20-39 have been withdrawn from consideration. New Claims 40-50 have been added.

A. Restriction

In paragraph 2 of the Office Action, the Examiner indicated that Claims 1-19 were drawn to a method of folding a polypeptide and that Claims 20-39 were drawn to a method of screening for an optimal folding environment for a denatured peptide. Applicant acknowledges the Examiner's restriction requirement and has withdrawn the second set of claims from Examination.

B. Drawings

In accordance with the Examiner's instructions in paragraph 4 of the Office Action, formal drawings will be filed once the application is allowed.

C. Information Disclosure Statement

Pursuant to Paragraph 8 of the Office Action, Applicant is re-submitting herewith an Information Disclosure Statement in accordance with the Examiner's request since one or more of the references appear to be missing from the file. Applicant respectfully submits that a bona fide attempt to comply with § 1.98 was made prior to the Final Office Action, but the references were either lost by the Patent Office or inadvertently omitted. As such, Applicant respectfully requests that pursuant to § 1.97(f), the Information Disclosure Statement be accepted. Applicant respectfully submits that the claims are patentable in view of the cited references.

D. Claim Rejections Under Section 102

1. Gorovitz Reference

In paragraphs 7-8 of the Office Action, the Examiner rejected Claims 1-3, 10 and 15-16 as being anticipated by the Gorovitz Article (1997). In response, Applicant notes that the claimed invention requires that the "chaperonin-polypeptide complex [be exposed] to an osmolyte, thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide." As such, the chaperonin must first be added prior to the addition of the osmolyte. The advantages of this two-step process are set forth in Applicant's specification:

> [T]he two-step folding procedure provides several important and unexpected benefits. The procedure combines the chaperonin's ability to prevent aggregation and even unfold the misfolded intermediates with the inherent structural stabilization and enhancement of folding afforded through the use of osmolytes. As the experiments with GSΔ468 demonstrate in Table 1, this combination can produce a remarkable synergistic amplification of protein folding in vitro. Because the refolding of denatured protein is performed in two steps; the solution parameters such as temperature, ionic strength, and protein concentration can be adjusted independently to insure both the efficient chaperonin-substrate complex formation and the optimal substrate release and refolding in the presence of osmolytes. The high stability of the complex allows for an easy manipulation of solution conditions without the significant loss of the folding proteins due to aberrant aggregation at higher concentrations. In the case of GSΔ468 , substrate concentration was initially kept low in order to avoid rapid aggregate formation and insure high chaperonin-to-substrate stoichiometry. Once the complex is formed, however, the substrate concentration can be increased to enhance the concentration-dependent second order GSΔ468 assembly reaction as shown in Table 2.

See Paragraph [0063] (emphasis added). Applicant has further amended independent Claim 1 to clarify the advantages of the present invention: namely, that the two step process results in "promoting [that] is greater than that which is achieved using chaperonins and osmolytes alone."

Because these limitations are neither taught or suggested by the Gorovitz Article, Applicant respectfully requests that the Examiner withdraw the rejection under Section 102.

In the Gorovitz Article, urea was used to denature the protein itself (equivalent to step (a) of Applicant's claimed invention), not to promote the folding of the polypeptide as required by the claimed invention. The Gorovitz Article indicates that the unfolded protein was then diluted with buffer and the chaperonin so that the urea concentration did not exceed 0.5M. Clearly, the urea (the purported osmolyte) was not added to the protein after the addition of the chaperonin.

The precise order of this chaperonin/osmolyte protein folding process is crucial to insure successful screening for optimal conditions. The folding protein is initially captured by the oligomeric chaperonin to form an arrested chaperonin-protein substrate complex. Applicant has repeatedly shown that this arrested form can hold to protein in a metastable but eventually foldable state for a long period of time (~2 hr at 37°C; Fisher, 1992). Once this complex is formed, the test osmolyte solution(s) can then be added to the arrested chaperonin-protein substrate complex and released into the test osmolyte solution where the ability of the osmolyte to influence successful protein folding can be evaluated. The order of addition is crucial because there are numerous instances where osmolyte addition to a folding substrate alone prior to forming the arrested chaperonin-protein complex results in large scale protein misfolding (Voziyan et al., 2000; Voziyan and Fisher, 2000). For example, Figures 4A and 6 in the latter reference show the glutamine synthetase misfolds (no activity, increase in aggregation species) when TMAO alone (without the chaperonin) is added to refolding glutamine synthetase monomers. Thus, to specifically test the applicant's procedure, it is necessary to first form the complex between protein (e.g. DHFR) and chaperonin (e.g. GroEL) and then add the test

osmolyte (e.g. urea) and ATP to determine if added osmolyte will allow the protein to acquire its correct folded structure once it is released from the chaperonin.

In short, the Gorovitz Article does not teach or suggest "exposing [the] chaperonin-polypeptide complex to an osmolyte" as required by the claims. Thus, for this reason also, Applicant requests that the Examiner withdraw the rejection under Section 102.

Further, the Gorovitz Article does not teach or suggest urea (the purported osmolyte) to promote folding. The prior art shows that GroEL actually suppresses the folding of dihydrofolate reducates ("DHFR"). See Viitanen et al., "*Complex Interactions between the Chaperonin 60 Molecular Chaperone and Dihydrofolate Reductase*," Biotechnology, at pp 9716-9723 (1991) (cited in the Gorovitz Article). GroEL only has the ability to release DHFR after ATP is added to the arrested DHFR-chaperonin complex. The released DHFR does not gain any advantage of folding yield with the chaperonin present because it does not require the chaperonin to fold unlike the particular protein substrate that was used (ΔV468 GS) in our original patent application.

The Gorovitz Article merely investigated whether differing denaturing conditions (urea vs. thermal) would change the chaperonin requirements (GroEL, GroES ATP vs. GroEL GroES ADP). The reference simply created a different denatured protein to bind to the chaperonin by using either heat or urea to denature the protein. The folding of the protein DHFR was initiated in the presence of GroEL with residual urea present. However, the inclusion of urea did not in any way promote the folding of DHFR to its native conformation as required by the claimed invention. See Claim 1 ("exposing said chaperonin-polypeptide complex to an osmolyte, thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide"). Further, there is not any teaching or suggestion that the

resulting "promot[ion] is greater than that which is achieved using chaperonins and osmolytes alone." As such, for this additional reason, Applicant requests that the Examiner withdraw the rejection under Section 102.

2. Altamirano References

In paragraphs 9 to 11 of the Office Action, the Examiner rejected Claims 1-3, 10, 13-15 as being anticipated under 35 U.S.C. § 102 by Altamirano (1997) or Altamirano (1999) (together the Altamirano Articles). As discussed above, the claimed invention requires that the chaperonin-protein complex must be formed prior to the addition of the osmolyte. Because the Altamirano Articles do not teach or suggest such a step, Applicant respectfully traverses the rejection.

In the 1997 Altamirano Article, the mini-chaperone was mixed with refolding buffer (2M KCl and 2 M urea). Next, the denatured protein (cyclophilin A in 8M urea as a denaturing agent) is added to the suspension. As such, the chaperonin-protein complex is not formed prior to the addition of the osmolyte as required by the claims. In other words, the there is no teaching of suggestion of exposing the "chaperonin-polypeptide complex to an osmolyte, thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide." Because this limitation is not taught or suggested by the 1997 Altamirano Article, Applicant respectfully requests that the Examiner withdraw the rejection under Section 102.

Likewise, in the 1999 Altamirano Article, the refolding buffer (containing potassium phosphate and arginine) is first mixed with the refolding gel (containing either (1) the binary matrix of DsbA and minichaperone or (2) the ternary matrix of DsbA, PPI, and minichaperone). The denatured scorpion toxin Cn5 is then added. See Fig. 2 (caption); Table 1 (caption). Again,

the chaperonin-protein complex is not first formed prior to the addition of the osmolyte as required by the claims. Because this limitation is not taught or suggested by the 1999 Altamirano Article, Applicant respectfully requests that the Examiner withdraw the rejection under Section 102.

Applicant has also added new independent Claim 40, which recites that the chaperonins of the present invention are "oligomeric." No new matter has been added by this amendment. See Paragraph [0065] of the specification (describing the oligomeric GroEL). In view of this Amendment, Applicant respectfully submits that the claimed invention is patentable over the cited references.

As discussed in Applicant's Amendment and Response of June 25, 2003, the claimed "oligomeric chaperonins" are not the same as the fragmented "minichaperone system" described in the cited Altamirano references. The minichaperone system is inferior to and will not function with most commonly used polypeptides. In this regard, the Wang (1998) and Weber (1998) articles included in the Information Disclosure Statement show that the Altamirano minichaperone system fails to fold various stringent protein substrates. In cases where successful folding with the minichaperone is observed, Altamirano and his coworkers refolded one stringent class III chaperonin dependent protein, but only under conditions where rodanese can fold by itself (so-called "permissive" 25° C folding conditions). See Wang (1998); Smith & Fisher (1995). Thus, it does not appear that the minichaperone was even required to fold this protein to any degree.

In particular, Wang (1998) and Weber (1998) showed that the minichaperone system could not function to fold malate dehydrogenase. In contrast, as illustrated in Example 8, the present invention was capable of working with other substrates (such as malate dehydrogenase).

See also (Tieman 2001). Further, subsequent work by the inventors (Voziyan 2000) indicates that the present invention involving chaperonin/osmolyte systems shows that it works with citrate synthase, another one of the test substrates that the minichaperones failed to fold. See Weber (1998). The primary difference between the claimed invention, which uses the oligomeric version of the chaperone, and that of the minichaperone fragment is that the latter cannot capture folding polypeptides or stabilize a metastable protein state for any length of time.

In short, there is nothing in the prior art that teaches the use of an osmolyte/"oligomeric chaperonin" system to promote the folding of a polypeptide. Applicant respectfully submits that the Altamirano references do not teach or suggest the claimed invention. As such, withdrawal of the rejection is requested.

In view of the foregoing amendments and remarks, it is respectfully submitted that the claims are now in condition for allowance and eventual issuance. Such action is respectfully requested. Should the Examiner have any further questions or comments in order to obtain allowance, he is invited to contact the undersigned attorney at the number listed below.

Acknowledgement of receipt is respectfully requested.

Respectfully submitted,

By: _____

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